**Periodic stripe formation by a Turing-mechanism operating at growth zones in the mammalian palate**

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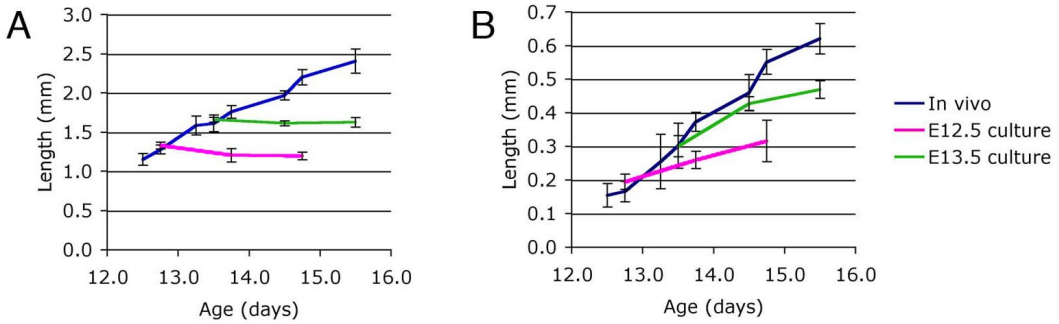
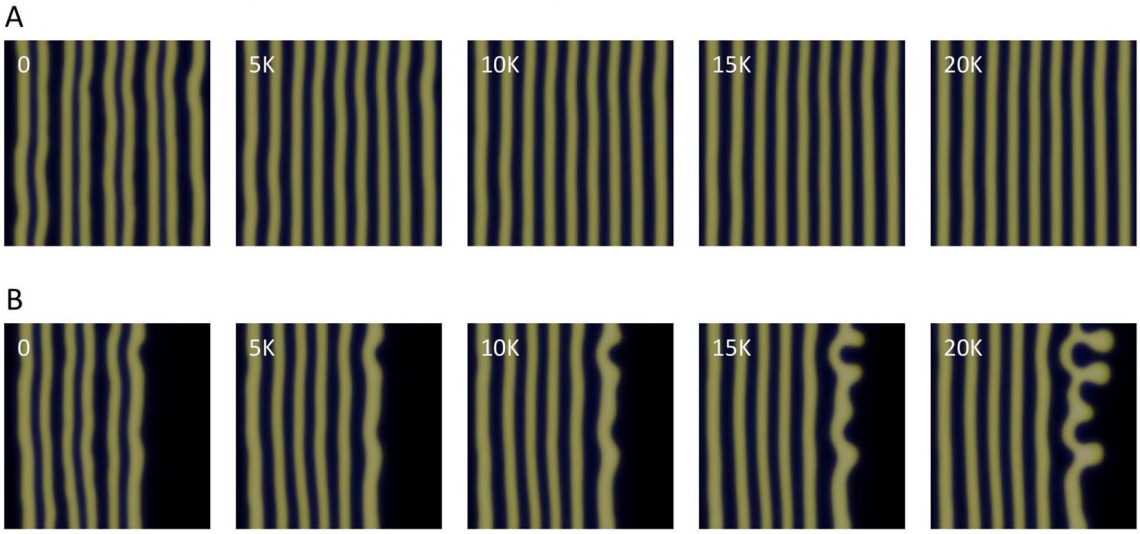
Supplementary Information:

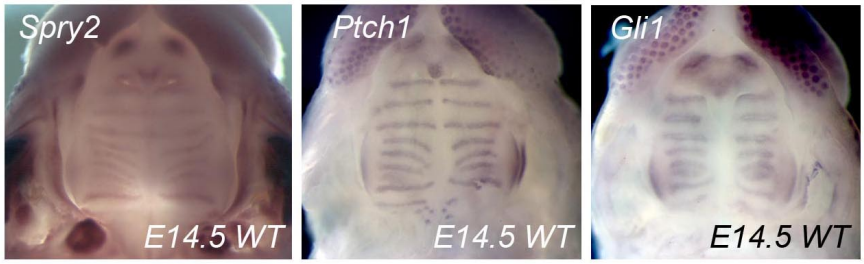
**Supplementary Figure S1 Palate explant culture arrests anteroposterior but not mediolateral growth.** (A) Overall AP length and (B) ML length of in vivo shelves (blue) plotted against age, compared to cultured shelves explanted on embryonic days 12.5 (magenta) and 13.5 (green). Shelves were cultured for 24 and 48 hours and plots indicate the lengths of shelves in littermates for two representative litters. While AP growth is arrested in culture (flat red and green plots in A), ML growth occurs in culture at a reduced rate (B). For in vivo plots samples of at least 19 palatal shelves were used for each point, and for cultured plots at least 6 shelves were used.

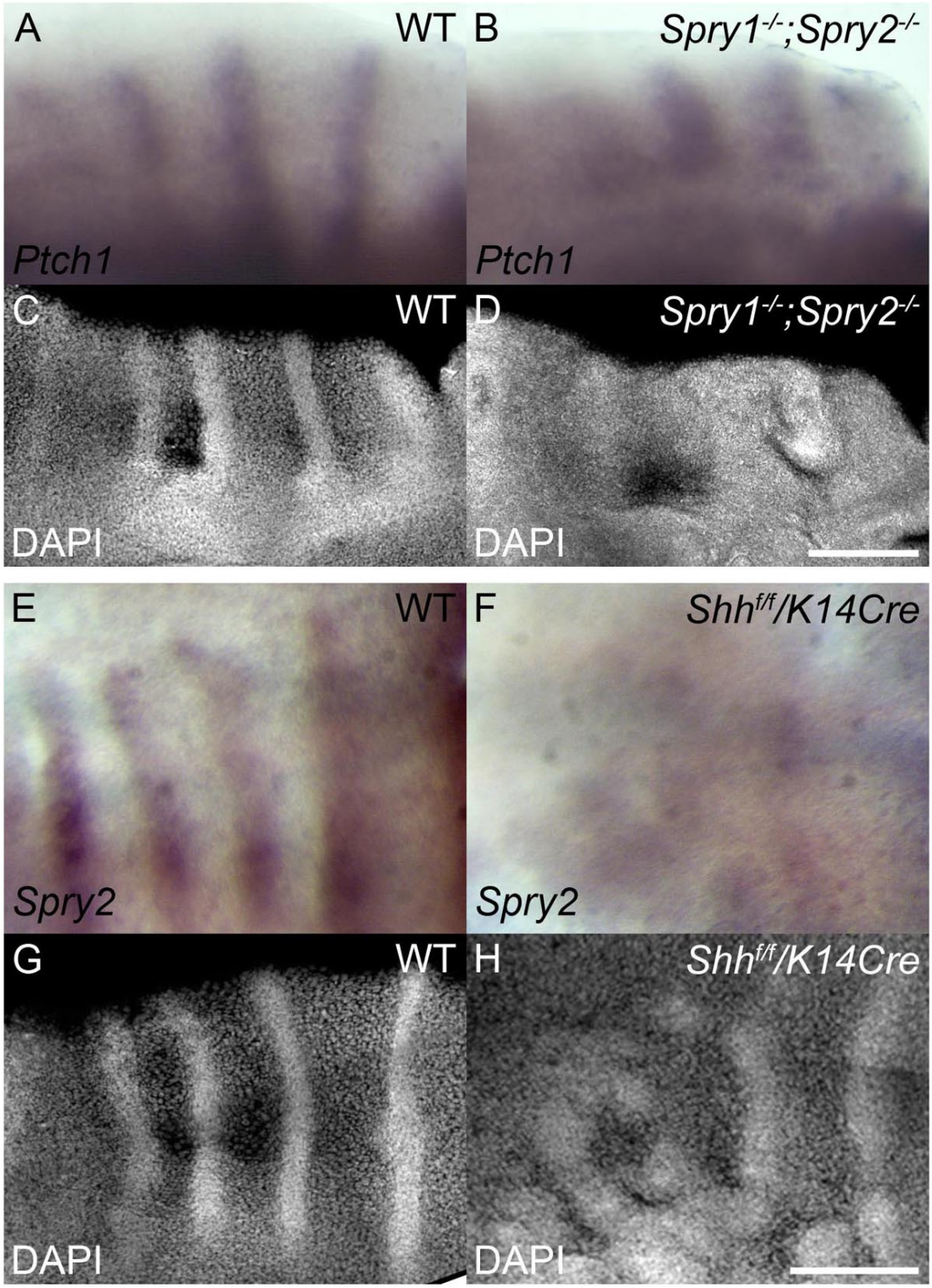



**Supplementary Figure S2 Branches arise upon stripe removal in reaction-diffusion simulations.** The figure shows time series of simulations using Turing equations as in ref. 2 with identical parameters in (A) and (B) but differing only in the presence or absence of three stripes at the right. Neighbouring stripes suppress deviations and branching (A) while upon removal of stripes (B), branches appear and grow sideways. Numbers indicate iterations of the model calculations (5K = 5,000 iterations, etc.). The simulation reveals qualitative features of a reaction-diffusion system and no attempt was made to match the proportions or scale of the live experiments.



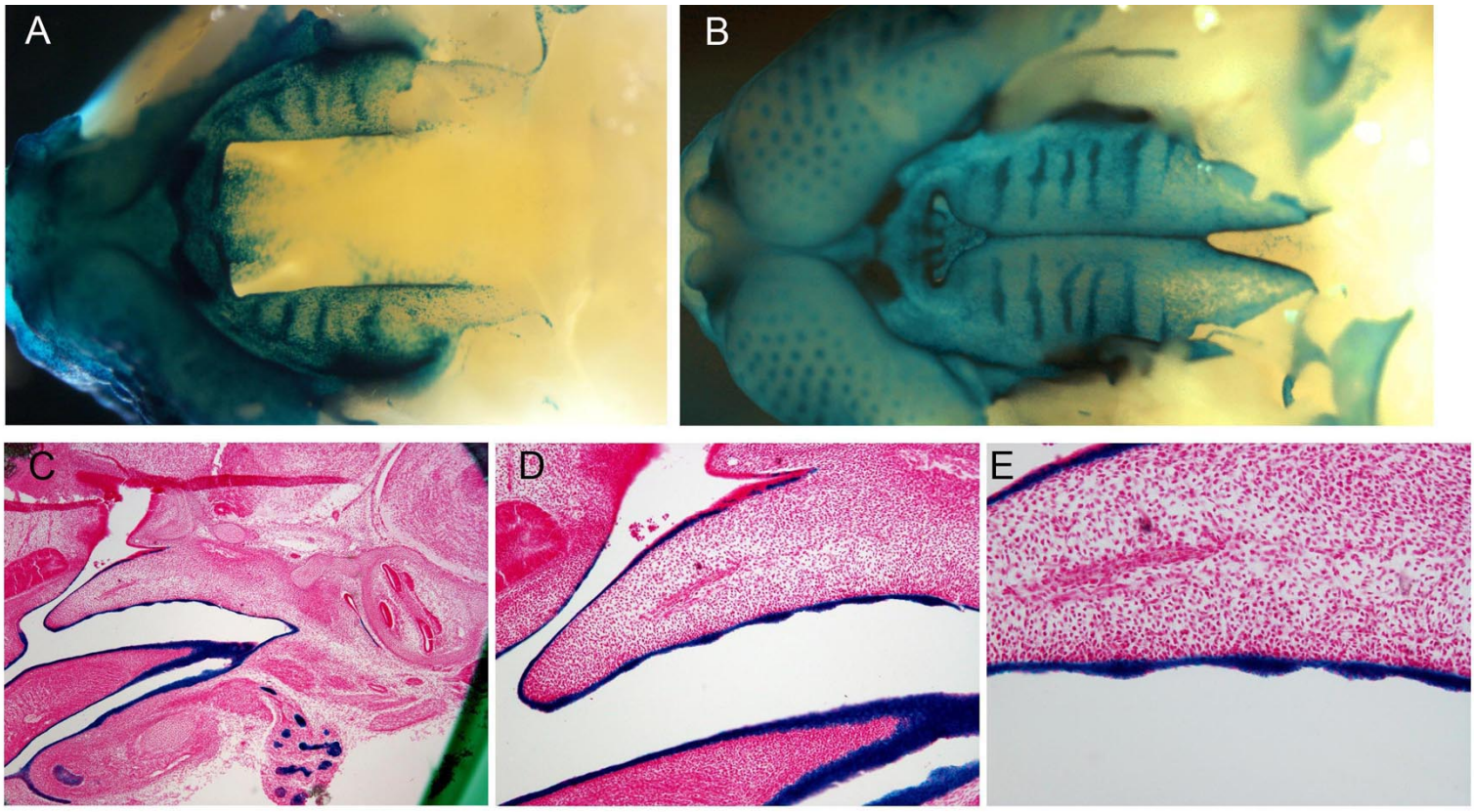

**Supplementary Figure S3 Expression of *Spry2*, *Ptch1* and *Gli1* in normal palates**. Palates viewed from the oral side, anterior up showing wholemount in situ hybridisations with probes for *Spry2, Ptch1* and *Gli1* show expression of these genes in rugae of E14.5 embryos.



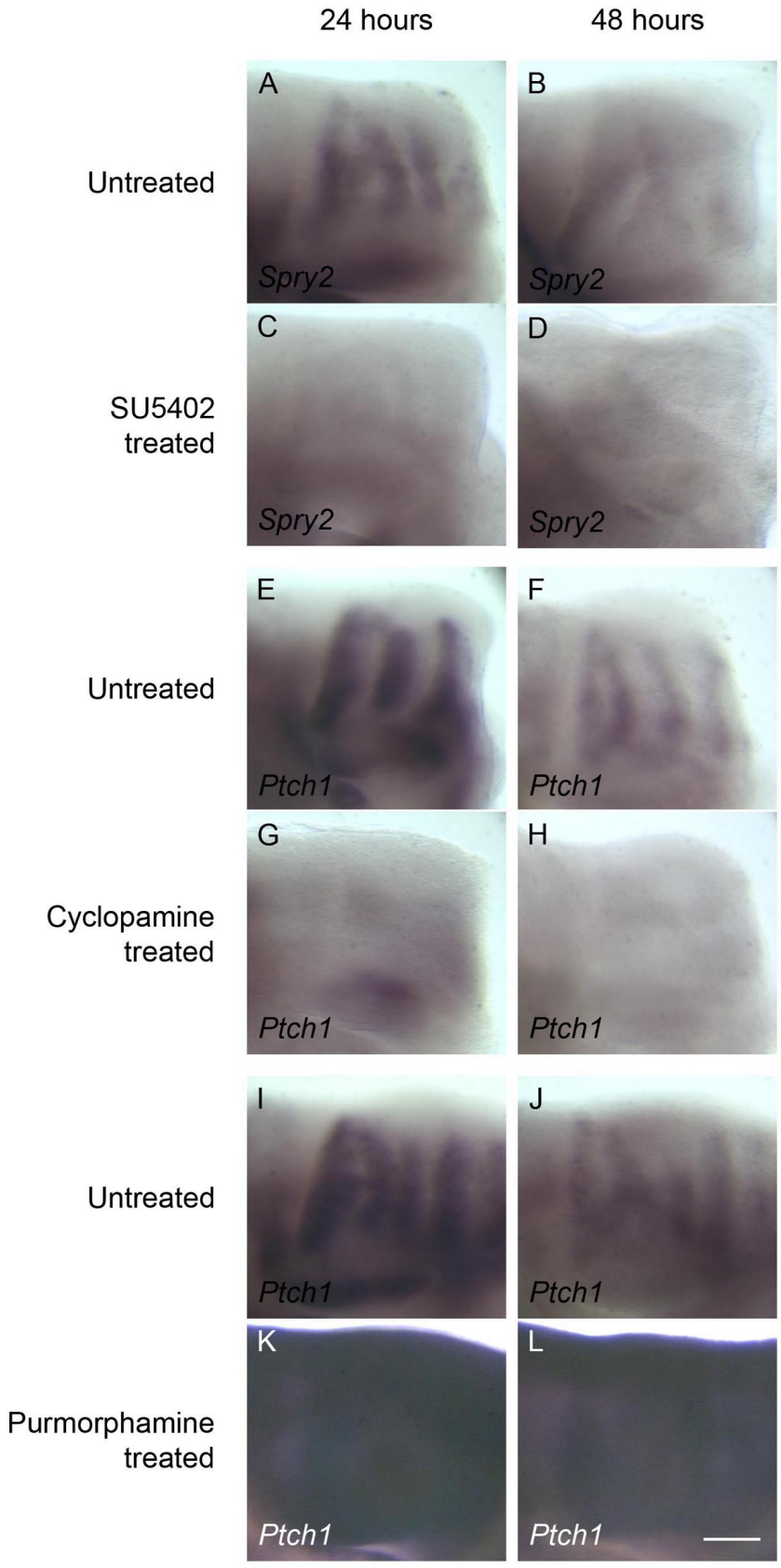



**Supplementary Figure S4 Disrupted patterns of *Ptch1* and *Spry2* expression mirror epithelial thickening and expanded, disorganised rugae in *Sprouty* and *Shh* mutant palates**.

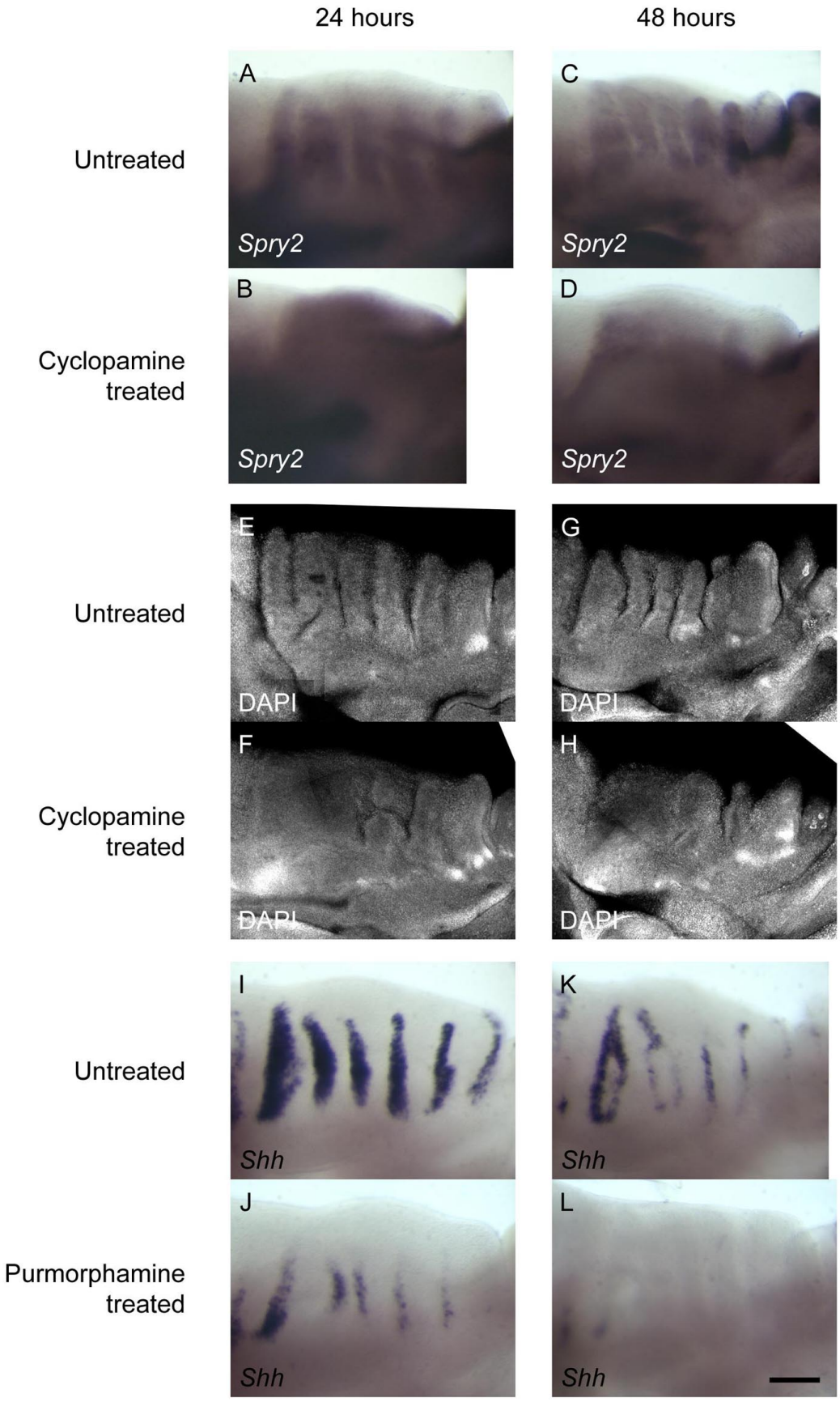
A, B. In situs for *Ptch1* expression show broadened broken stripes in *Sprouty* double null mutants (B) compared to controls (A). C, D. Maximum projections of z-stacks of confocal images of palates stained with the nuclear stain DAPI reveal early rugal epithelial thickening in normal stripes and broadened, broken stripes in wild type (C) and Sprouty double null mutant (D palates respectively. E, F. In situs for *Spry2* expression show broadened broken stripes in *K14-Cre/Shh*$^{fl/fl}$ mutants (F) compared to controls (E). G,H. Maximum projections of z-stacks of confocal images of palates stained with the nuclear stain DAPI reveal early rugal epithelial thickening in normal stripes and broadened, broken stripes in wild type (G) and *K14-Cre/ Shh*$^{fl/fl}$ mutant (H) palates respectively. Scale bars = 200 μm.




**Supplementary Figure S5 *K14-Cre* drives recombination throughout the palate epithelium.**
(A, B) Whole-mount β-galactosidase staining portraying cells that underwent Cre recombination events (dark blue) in the palate epithelium of (A) E13.5 and (B) E14.5 *K14-Cre/ROSA26-lacz* mouse embryo. (C-E) Representative parasagittal cryostat section (12 µm-thick) of an E14.5 *K14-Cre/ROSA26-lacz* mouse embryo showing β-galactosidase staining (dark blue) throughout the epithelium. Original magnifications: x4 (C), x10 (D), x20 (E). Note wholemount staining is always much more granular than in sections and so could be staining artefact or indicator of mosaic recombination rate.




**Supplementary Figure. S6 Controls showing efficacy of SU5402, cyclopamine and purmorphamine.** Palate explants were cultured in the presence of SU5402,cyclopamine or purmorphamine for 24 or 48 hours and probed for expression of *Spry2* as a marker of FGF signalling or *Ptch1* as a marker of Hedgehog signalling as shown. SU5402 inhibited *Spry2* expression (C,D), cyclopamine inhibited *Ptch1* expression (G,H) while purmorphamine massively stimulated *Ptch1* expression (K,L) compared to sibling controls (A,B,E,F,I,J), as expected. The slightly green colour in panels K and L is a byproduct of enhancing the Brightness of the image needed to show the slight variation in staining across the tissue. Palates are shown anterior to right, medial up. Scale bar = 200 μm.




**Supplementary Figure S7 Cyclopamine broadens *Spry2* and epithelial thickening stripes while Purmorphamine inhibits *Shh* stripes** (A, C) In situ hybridisation for *Spry2* showing expression is localised to the rugae after 24 and 48 hours in culture. (B, D) In situ hybridisation for *Spry2* showing expression in cultures treated with Hedgehog signalling inhibitor cyclopamine showing expanded expression compared to untreated explants. (E-H) Maximum projections of image z-stacks of confocal images of palates stained with the nuclear dye DAPI showing controls with well-organised stripes of epithelial thickening (E,G) and expanded and disorganised thickening in cyclopamine-treated palate explants (F, H). (I-L) In situ hybridisation for *Shh* showing striped expression in controls (I, K) is inhibited following treatement with the Hedgehog signalling agonist purmorphamine (J,L). Palates are shown anterior to right, medial up. Scale bar = 200 μm.